Physiological responses of *Ulva rigida* C. Agardh, to nutritional and growth substances

*Ulva rigida* C. Agardh occurs at Gopnath near Bhavnagar, on sand stone rocks throughout the intertidal zone reaching maximum growth in rock pools. It has been found that *Ulva* spp. are rich in protein and nutritionally important with regard to amino acid composition. Of all the species studied it has been shown that *Ulva rigida* C. Ag. contains all the essential amino acids.

The species is restricted to Gopnath and is not found in harvestable amount. Hence there is an urgent necessity to evolve a method of cultivation of this alga. Towards this end and to develop a possible fertilizer for the cultivated seaweed it is essential to study the nutritional requirements of this alga. Moreover, it is necessary to investigate the growth of the alga under different conditions in culture as well as in the field.

In these investigations, the growth of the alga from swarmers into the germlings under conditions of culture and also the growth of excised pieces of the thallus of the alga in different nutrient concentrations as well as concentrations of growth promoting substances have been studied in detail.
Studies on germlings:

Swarmers could be readily obtained by cutting out fragments of mature thalli and placing them either in fresh filtered sea water or in Erd-Schreiber sea water and these were observed to germinate readily under laboratory conditions, giving rise to short filamentous sporelings. The rate of growth was much higher in Erd-Schreiber than in plain sea water. An attempt was also made to grow the sporelings in synthetic sea water. After some stages the growth of the sporelings became abnormal.

One month old germlings were also treated with the commercial fertilizers, potassium phosphate (as source of phosphorus) and ammonium sulphate and urea (as sources of nitrogen). Ammonium sulphate enhanced the growth of germlings at very low concentrations while urea gave very good results over a wide range of concentrations from 5 mg. to 15 mg. %. Potassium phosphate gave rise to very small increase in growth at low concentrations, while it was toxic at higher concentrations.

Studies on excised pieces:

The growth of excised pieces from various regions of Ulva was studied in the laboratory and it was found that there was a definite pattern of growth. The apical region of the thallus constitutes the region of elongation, while middle, midbasal and marginal regions constitute the regions of areal expansion. The basal region shows comparatively little growth.
To confirm whether this pattern of growth is due to distribution of growth hormones, the above study was repeated, but by first treating the excised pieces from different regions by a known concentration of indole acetic acid. It was seen that the addition of indole acetic acid had no further effect on the apical region but that the growth was enhanced in the marginal and middle regions. The results indicate that the apical region already had an optimum level of hormone while other regions had lower levels of the hormone.

In subsequent experiments on the effect of fertilizer chemicals and growth substances on the growth of excised pieces, only fragments removed from the medium portion of vigorously growing vegetative thallus of more or less uniform state of development were used.

It was found out that a photoperiod of sixteen hours gave good vegetative growth of the excised pieces in contrast to continuous illumination.

Three different sources of phosphorus, viz. di-potassium hydrogen phosphate, di-sodium hydrogen phosphate and sodium glycerophosphate were used to determine the best source of phosphorus for the growth of the alga. It was found that Ulva can utilise both inorganic and organic phosphates. Di-potassium hydrogen phosphate was weakly effective and only at low concentration. Glycerophosphate produced very good growth but was effective only at low concentration upto
2 mg%. Beyond this level it was toxic. Di-sodium hydrogen phosphate produced very good growth over a wide range of concentrations.

Nitrogen was supplied in the form of following substances: Sodium nitrite, sodium nitrate, ammonium sulphate, urea and asparagine. Nitrite and ammonium sulphate were not good sources of nitrogen, although at low concentrations, ammonium sulphate gave some enhancement in growth. Nitrate was effective over a wide range of concentrations upto 25 mg%, beyond which it was toxic; urea and asparagine gave the best results, the former being effective over a range of concentrations from 5 to 15 mg% while the later at a low concentration of 5 mg%.

The response of the excised pieces to different growth substances was studied in culture both under continuous illumination and under a photoperiod of sixteen hours using molar concentrations of indole acetic acid, and \( \alpha \)-naphthyl acetic acid, gibberellic acid, adenine and kinetin. It was observed that the pieces do not respond to indole acetic acid under continuous illumination, while under photoperiod, a high increase in growth over the control was seen. The excised pieces respond to the other growth substances viz. gibberellic acid, naphthyl acetic acid, adenine and kinetin both under continuous illumination and under photoperiod.

The optimum level of growth substances required by *Ulva rigida* was low, \( 10^{-7} \) M indole acetic acid, \( 10^{-7} \) M \( \alpha \)-naphthyl acetic acid, \( 10^{-5} \) M to \( 10^{-6} \) M gibberellic acid,
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$10^{-7} \text{ M adenine and } 10^{-8} \text{ M kinetin}$. Concentrations higher than these proved toxic to the alga.

Throughout the experimental period the treated excised pieces or sporelings of *Ulva rigida* showed a purely vegetative growth.

From the above investigations following conclusions can be drawn:

1. It has been clearly demonstrated that the growth in the thallus of *Ulva rigida* is not diffused but that there is a definite pattern of growth and this closely follows a pattern of distribution of growth hormones in the thallus.

2. A new approach has been made to study the nutritional requirements of *Ulva rigida*, by using excised pieces from the most vigorously growing part of the thallus and valuable information has been obtained with reference to requirements of phosphorus, nitrogen and growth promoting substances for maximum vegetative development in the alga.

3. It has been shown that *Ulva rigida* can utilize inorganic as well as organic compounds as sources of phosphorus and urea and asparagin as sources of nitrogen. Growth substances, indole acetic acid, $\alpha$-naphthyl acetic acid, gibberellic acid, adenine and kinetin are utilised in low levels.

4. A photoperiod of sixteen hours gives better growth of *Ulva* in all experiments. Continuous illumination retards growth in pieces treated with indole acetic acid.
From the above results, it is possible to formulate nutritional needs of *Ulva rigida* for purposes of culture and cultivation of the alga.